



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>6</sup> :</b> <b>A61K 31/35</b>	<b>A1</b>	<b>(11) International Publication Number:</b> <b>WO 98/56373</b> <b>(43) International Publication Date:</b> 17 December 1998 (17.12.98)
<b>(21) International Application Number:</b> PCT/US98/10605 <b>(22) International Filing Date:</b> 26 May 1998 (26.05.98)  <b>(30) Priority Data:</b> 08/873,314 11 June 1997 (11.06.97) US  <b>(71)(72) Applicant and Inventor:</b> GORBACH, Sherwood, L. [US/US]; 31 Perry Lane, Weston, MA 02193 (US).  <b>(74) Agents:</b> CLARK, Paul, T. et al.; Clark & Elbing LLP, 176 Federal Street, Boston, MA 02110 (US).		<b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i>
<b>(54) Title:</b> ISOFLAVONOIDS FOR TREATMENT AND PREVENTION OF AGING SKIN AND WRINKLES  <b>(57) Abstract</b>  A method of treating or preventing, in a person, one or more symptoms of aging skin, said method comprising topically administering to the skin of said person a composition comprising one or more isoflavonoids selected from the group consisting of genistein, daidzein, biochanin A, formononetin, O-desmethylangolensin, glycitin, and equol, in a topically acceptable base, wherein the isoflavonoid concentration is between 1 and 40 mg per gram of base.		

*FOR THE PURPOSES OF INFORMATION ONLY*

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Larvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

## ISOFLAVONOIDS FOR TREATMENT AND PREVENTION OF AGING SKIN AND WRINKLES

5

### BACKGROUND OF THE INVENTION

The present invention relates to therapies for the prevention and treatment of aging skin and wrinkles.

It has long been recognized that as people grow older, significant changes occur in their skin, specifically thinning, deepening of facial creases (wrinkling), and increased extensibility and flaccidity. These changes are related to reduced skin tonicity and diminished skin hydration. The underlying causes for these changes are believed to be lowered collagen content and reduced number of elastic fibers in the skin. Estrogen hormones have been used for treating aging skin either in an oral form or as topical skin creams or gels. These treatments have produced augmented skin thickness, greater hydration, and improvements in elasticity and firmness. It is believed that the effectiveness of estrogen hormones is related to the increase in the amount of skin collagen which is caused by stimulating collagen synthesis. Besides being able to demonstrate the increase in collagen content after estrogen treatment, there is also an increase in collagen and elastic fibers, which improve the mechanical properties of skin. While estrogen can be used for treating and preventing aging skin, potential users of this hormone are concerned about the risk of side effects, particularly the increased risk of cancers of the breast and uterus. In addition, estrogen typically is not used in men, who also have problems with aging skin and wrinkles, because of the undesirable side effects of this female hormone in male users. Safer and effective therapies for treating and preventing aging skin and wrinkles in both women and men continue to be sought.

-2-

## SUMMARY OF THE INVENTION

The invention features the topical use of purified isoflavonoids, which are constituents of soy beans and other plants such as clover, to effectively treat and prevent symptoms of aging skin, such as wrinkles. Without being bound by any theory, it is believed that isoflavonoids have significant estrogenic activity, acting in the skin by stimulating the synthesis of collagen. These compounds are safe and cause no significant side-effects. Purified isoflavonoids which may be administered according to the invention include genistein, daidzein, biochanin A, formononetin, O-desmethylangolensin, glycitin, and equol; these may be administered alone or in combination.

Accordingly, the invention provides a method of treating or preventing, in a person, one or more symptoms of aging skin, e.g., wrinkles, by applying to the person's skin a composition containing a dermatologically acceptable base containing between 1 and 40 mg purified isoflavonoid per gram of base; the isoflavonoid is one of the naturally-occurring isoflavonoids listed above.

By "purified" is meant the isoflavonoid is in a form which is more concentrated than the form in which it occurs naturally in plants.

Preferred topical formulations are creams, ointments, lotions, emollient creams and ointments, moisturizing lotions, and gels. The purified isoflavonoids can also be included in a transdermal delivery system or patch.

The purified isoflavonoids of the invention can also be included in cosmetics (e.g., makeup); preferred forms are lotions, creams, moisturizing creams and lotions, skin oils, skin sprays, and gels.

Preferably, the topical composition containing the purified isoflavonoids is applied to the skin once or twice per day.

Alternatively, the invention features a method for treating or preventing one or more symptoms of aging skin in a male person or a female person three

-3-

or more years past the onset of menopause, by administering (preferably orally) to the person a composition containing one or more purified isoflavonoids selected from the group consisting of genistein, daidzein, biochanin A, formononetin, O-desmethylangolensin, glycitin, and equol, in an amount

5 sufficient to produce a transient concentration of the bloodstream of the person of at least 50 nm/l. Preferably, the composition is administered orally, providing a dosage of at least 20 mg of total isoflavonoid per serving. The orally-administerable composition can be a non-naturally occurring dietary product such as a confectionary bar, cereal, biscuit, or beverage. Alternatively,

10 the composition can take the form of a medicament such as a pill, capsule, tablet, powder, or syrup, in which the total isoflavonoid is present in at least an amount of 20 mg per unit dose. Preferably, the composition provides a dosage of at least 20 mg of total isoflavonoid per serving. The orally-administerable composition can be a non-naturally occurring dietary product such as a

15 confectionary bar, cereal, biscuit, or beverage. Alternatively, the composition can take the form of a medicament such as a pill, capsule, tablet, powder, or syrup, in which the total isoflavonoid is present in at least an amount of 20 mg per unit dose. Preferably, the dietary product or medicament is orally consumed by the person once, twice, or three times per day, to provide a daily

20 oral isoflavonoid dose of between 20 and 300 mg. Preferably, the oral ingestion of the composition is sufficient to produce a transient concentration in the bloodstream of the person of at least 50 nm of total isoflavonoid per liter of blood. By "purified" isoflavonoid is meant an isoflavonoid in more concentrated form than occurs in plants.

25 Other features and advantages of the invention will be apparent from the Detailed Description thereof, and from the claims.

### DETAILED DESCRIPTION

Isoflavonoids are naturally occurring compounds, found primarily in soy beans. These compounds are also found in high concentrations in red clover and in lower amounts in many other types of plants. An isoflavonoid-  
5 containing fraction (containing purified isoflavonoids) useful in the invention can be extracted from a soy or plant product using known methods. It is preferred that the isoflavonoids be extracted and concentrated from soy beans or soy powder, but other plants such as clover can be used. Isoflavonoids are also available commercially in substantially pure form.

10 The purified isoflavonoid, in the dermatologically acceptable base, is applied directly to the skin surface. The topical composition should be left on the skin for a sufficient period of time to allow the isoflavonoid to be substantially absorbed into the skin and the capillaries supplying the skin; generally, this period of time should be at least one, and preferably at least  
15 three hours. Where the topical composition is a cosmetic, it can be removed in the manner of ordinary cosmetics, e.g., using "cold cream." Because the isoflavonoids are not toxic, the topical composition can be applied at bedtime and left on the face, or other skin surface, overnight.

The isoflavonoid-containing composition can also be included in a  
20 transdermal delivery system or patch. The transdermal patch can be of conventional form, e.g., that used to deliver sustained doses of nicotine or estrogen.

Isoflavonoids have similar chemical properties to estrogens, e.g., they are poorly soluble in water but are readily soluble in alcohols and other organic  
25 solvents. For topical applications, either as a medicament or incorporated into a cosmetic, isoflavonoid is mixed in a base with ingredients such as alcohol, mineral oil, glyceryl monostearate, ether complex of fatty acids, cetyl alcohol,

-5-

lanolin, propylene glycol, stearyl alcohol, and sodium lauryl sulfate. The concentration of isoflavonoid is 1 to 40 mg per gram of the base, more preferably 10-25 mg per gram of base.

Other embodiments are within the claims.

-6-

We claim:

1. A cosmetic composition for application to the surface of the skin of a person for prevention or treatment of symptoms of aging skin, said cosmetic composition comprising a dermatologically acceptable base containing between  
5 1 and 40 mg per gram of base of one or more purified isoflavoids selected from the group consisting of genistein, daidzein, biochanin A, formononetin, O-desmethylangolensin, glycitin, and equol.
2. The composition of claim 1, wherein said composition is in the form of a cream ointment, lotion, emollient cream or ointment, moisturizing lotion,  
10 or gel.
3. The composition of claim 1, wherein said composition is a cosmetic.
4. The composition of claim 3, wherein said cosmetic is a lotion, cream, moisturizing cream or lotion, oil, skin spray, or gel.
5. The composition of claim 1, wherein said composition is carried on a  
15 transdermal delivery system or patch.
6. Use of one or more purified isoflavonoids selected from the group consisting of genistein, daidzein, biochanin A, formononetin, O-desmethylangolensin, glycitin, and equol in the preparation of a medicament for treating or preventing one or more symptoms of aging skin in a male human  
20 or a female human who is three or more years past the onset of menopause.
7. The use of claim 6, wherein said composition is formulated to be

-7-

administered orally, in a dosage of at least 20 mg of isoflavonoid per serving.

8. The use of claim 6, wherein said composition is in the form of a non-naturally occurring dietary product.

9. The use of claim 8, wherein said produce contains at least 20  
5 mg/serving of said isoflavonoid.

10. The use of claim 8, wherein said dietary product is a confectionary bar.

11. The use of claim 8, wherein said dietary product is a cereal.

12. The use of claim 8, wherein said dietary product is a biscuit.

10 13. The use of claim 8, wherein said dietary product is a beverage.

14. The use of claim 6, wherein said composition is in the form of a medicament.

15. The use of claim 14, wherein said composition contains at least 20 mg/unit dose of isoflavonoid.

15 16. The use of claim 14, wherein said medicament is in the form of a pill, capsule, tablet, powder, or syrup.

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US98/10605

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : A61K 31/35  
US CL : 514/456

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/456

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y,P	US 5,603,936 A (MONTE) 18 February, 1997, column 1, lines 5-16; examples 26-37.	1-16
Y	US 4,218,489 A (ZILLIKEN) 19 August, 1980, column 2, lines 32-35; column 5, lines 19-37; column 6, lines 34-45.	1-16
Y	US 5,539,129 A (ZYSAMAN et al.) 23 July, 1996, column 7, lines 30-66; column 8, lines 1-65	1-16
Y,P	US 5,654,011 A (JACKSON et al.) 05 August, 1997, column 4, lines 51-67; column 5, lines 1-14; column 7, lines 50-67; column 8, lines 1-17.	7-16



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
*A* document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
*B* earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
*L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*G* document member of the same patent family
*O* document referring to an oral disclosure, use, exhibition or other means	
*P* document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

20 JULY 1998

Date of mailing of the international search report

03 SEP 1998

Name and mailing address of the ISA/US  
Commissioner of Patents and Trademarks  
Box PCT  
Washington, D.C. 20231

Facsimile No. (703) 305-3230

Authorized officer

LAKSHMI S. CHANNAVAJALA

Telephone No. (703) 308-1235

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION  
International Bureau



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification 6 :</b> <b>A61K</b>	<b>A2</b>	<b>(11) International Publication Number:</b> <b>WO 97/46208</b> <b>(43) International Publication Date:</b> 11 December 1997 (11.12.97)
<b>(21) International Application Number:</b> PCT/US97/11963 <b>(22) International Filing Date:</b> 9 June 1997 (09.06.97)  <b>(30) Priority Data:</b> 08/657,915      7 June 1996 (07.06.96)      US  <b>(71) Applicant:</b> MT. SINAI SCHOOL OF MEDICINE OF THE CITY OF NEW YORK [US/US]; One Gustave L. Levy Place, New York, NY 10028-6574 (US). <b>(72) Inventor:</b> WEI, Huachen; 114-15 Union Turnpike, Forest Hills, NY 11375 (US). <b>(74) Agent:</b> CLARK, Richard, S.; Brumbaugh, Graves, Donohue & Raymond, 44th floor, 30 Rockefeller Plaza, New York, NY 10112 (US).		<b>(81) Designated States:</b> AU, CA, GB, IL, JP, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).  <b>Published</b> <i>Without international search report and to be republished upon receipt of that report.</i>
<b>(54) Title:</b> GENISTEIN AS A PREVENTIVE AGAINST ULTRAVIOLET INDUCED SKIN PHOTODAMAGE AND CANCER  <b>(57) Abstract</b> <p>A method of inhibiting the harmful effect of UVR exposure to the human skin comprising topically applying a therapeutically effective amount of genistein to the skin at a time sufficiently close to the time of UVR exposure to inhibit UVR-induced damage to the skin. The genistein appears to act as a chemo preventative agent since it has no appreciable sun blocking effect. The genistein may be mixed with a variety of carriers and skin treatment compositions.</p>		

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakhstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

## Description

### Genistein as a Preventive Against Ultraviolet Induced Skin Photodamage and Cancer

#### Background of the Invention

The present invention relates to the prevention or treatment of skin damage and skin cancer, and in particular to ultraviolet radiation (UVR)-induced skin photodamage and cancer.

It is well documented that long term exposure to ultra-violet light, e.g., sunlight, will damage the skin. Such UVR-induced skin damage includes premature aging, as well as various skin cancers, for example, basal cell carcinoma, squamous carcinoma and malignant melanoma.

While the damaging effects of UVR are known, the recreational and occupational exposure to UVR is a day-today fact of life. While some people successfully minimize their exposure, others are either unwilling or unable to do so. For these people, exposure was either accepted as a risk, however unreasonable, or at best was prevented with various heretofore known sun blockers.

Sunscreens or sun blockers physically block the UV rays and thereby lessen the amount of UV light that would otherwise reach the skin. Known products include para aminobenzoic acid (PABA) as well as certain metal oxides. The evaluation of such products is discussed in Harry's Cosmeticology, Seventh Ed., pp. 222-263.

The above use of UVR blocking agents is to be distinguished from the use of chemopreventative agents against cancer or skin degenerative processes. Chemopreventative agents function to prevent or alter the various cellular or molecular carcinogenic processes that ultimately lead to tumor growth and the like.

The typical chemopreventative agents, on the other hand, must be administered into the body, such as by oral ingestion, or by injection.

With respect to orally ingested anti-cancer agents, epidemiological studies have shown that consumption of soybean-containing diets have been associated with lower incidence of certain types of human cancers. In particular, the soybean isoflavone "genistein" has been associated with the chemoprevention of cancer. See Wei et al., Inhibition of tumor promoter-induced hydrogen peroxide production in vitro and in vivo by genestein, Nutrition and Cancer 20:112, 1993; Antioxidant and Antipromotional Effects of the Soybean Isoflavone Genistein, Wei et al., Proceedings of society for Experimental Biology and Medicine, 208: 124-130, 1995.

The purification of genistein from soy products, e.g., soy molasses, is known. See Peterson et al., Genistein inhibition of the growth of human breast cancer cells; independence from estrogen receptors and the multidrug resistance gene, Biochem Biophys Res Commun 179: 6616671 1991, incorporated herein by reference.

#### Summary of the Invention

In accordance with the present invention, it has been discovered that genistein may be used as a topical chemopreventative agent against the adverse effects of UVR on the skin. Genistein may be topically applied, alone or co-administered with other medications, to lessen or prevent UVR-induced skin sunburns, premature aging, and skin cancer.

Genistein is uniquely suitable as a topically applied chemopreventative agent in that it is a natural product having no observed adverse effects or toxicities in humans. The physical and chemical properties of genistein are appropriate for a topical skin agent, i.e., it has high lipid solubility and easily penetrates the skin.

Data suggests that genistein exhibits potent and stable antioxidant activities. It scavenges reactive oxygen species and increases antioxidant enzymes in mouse skin tissue. Thus it may serve to delay skin aging and inhibit skin tumorigenesis.

Since genistein also relieves chemical carcinogen-induced skin inflammation, it may serve as an antiinflammatory agent on chemical skin irritations.

Genistein's ability to inhibit chemical carcinogen-induced protooncogene expression and tumorigenesis permits its use as a chemopreventive agent against chemical-induced carcinogenesis of skin. Genestein's ability to quench UVR-induced oxidative DNA damage in vitro and in cell culture makes it a useful topical agent for inhibiting the initiation of skin photocarcinogenesis. Moreover, genistein has been shown to suppress UVR-induced protooncogene expression (i.e., in mouse skin) and phosphorylation of the epidermal growth factor receptor in human keratinocytes, thus indicating genistein's ability to inhibit the promotion of skin photocarcinogenesis.

While the possible UVR blocking effect of genistein cannot be entirely discounted, it is genistein's chemopreventive properties that are of particular interest. Thus while the use of a conventional sun-blocking product connotes exactly what the name implies i.e., that the sun actually be blocked from reaching the skin -- a chemopreventative product need only be typically applied in such a manner that the chemopreventative mechanism function in a therapeutically effective manner.

It is therefore contemplated that such compositions be applied even on the assumption that otherwise harmful UVR or chemical agents will have reached the skin. Included would be the topical application before, during or even after exposure to UVR or other harmful agents, so long as the desired chemopreventive effect can take place to a therapeutically effective extent.

Compositions according to the invention can also be combined with compositions that have other UVR-blocking or antiaging properties. They can also be combined with carriers that will facilitate penetration into the skin, such as DMSO, ethanol, propylene glycol, etc. Finally, the compositions can be combined with compositions that have other cosmetic or medicinal properties, such as skin creams, make-up preparations, tanning lotions or the like.

As noted above, various properties, effects and mechanisms of genistein have been disclosed by the present inventor, although not necessarily as part of the prior art. The above-referenced publications, as well as the prior art and non-prior art publications cited therein, are incorporated herein by reference for purposes of providing background. While some of the properties or mechanisms discussed therein may provide some explanation of the beneficial effects obtained according to the presently disclosed topical uses of genistein, other mechanisms or combinations of mechanisms may be involved.

#### Brief Description of the Drawings

The description of the preferred embodiments is further explained below with reference to the figures, wherein:

Figure 1 is a photograph of a patient's back after UVB exposure as described in Example 1.

Figure 2 is a photograph of the gel electrophoresis as described in Example 3.

Figure 3 is a photograph of the gel electrophoresis as described in Example 4.

Figures 4 and 6 are photographs of the gel electrophoreses as described in Example 5.

Figure 5 is a graph showing the quantitation of transcript levels, also as described in Example 5.

#### Detailed Description of the Preferred Embodiment

Various topical uses of genistein are contemplated by the present invention. These include the prevention or treatment of the affects of UVR on the skin, e.g. premature aging and cancer. Also contemplated is the topical application of genistein as an antiinflammatory agent for chemical skin irritation. These uses are discussed in conjunction with the following examples.

#### Example 1

An experiment was performed to measure the effect of genistein on UV-induced skin erythema.

A human subject was subjected to UVB doses ranging from 0 to 90 mJ/cm<sup>2</sup> in three separate "lanes." These Lanes are depicted in Figure 1.

In Lane 1, the patient was first topically treated with a 5  $\mu$ mol solution of genistein per cm<sup>2</sup> of skin. The genistein was applied in a 5:95 DMSO:acetone carrier.

In Lane 2, no pre-treatment was given.

In Lane 3, the patient was topically treated with only the DMSO:acetone carrier.

As can be seen from Figure 1, Lane 1 shows virtually complete protection against skin erythema with the genistein. Both of Lanes 2 and 3 (i.e., no treatment; carrier only) showed UVB dose-dependent induction of skin erythema.

While the mechanism by which genistein inhibited erythema is unknown, the mechanism appears to be independent of the "sunscreen" effect. This was confirmed by dissolving up to 100 MM genistein in water. No blocking effect of UVB was observed in the genistein solution as compared to the water alone. Methods for testing the sunscreen effect of various compositions are discussed in Harry's Cosmeticology, Id.

#### Example 2

An experiment was conducted to determine the effect of genistein dosage on skin erythema inhibition.

The subject was uniformly exposed to a UVB dose of 45 mJ/cm<sup>2</sup>. The genistein dosage was varied from a high of 5  $\mu$ mol to a low of 0.0  $\mu$ mol per cm<sup>2</sup> of human skin (i.e., 0.0  $\mu$ mol; 0.05  $\mu$ mol; 0.1  $\mu$ mol; 0.5  $\mu$ mol; 1.0  $\mu$ mol; 5.0  $\mu$ mol). A striking inhibition of erythema was observed at the 0.1  $\mu$ mol level and above. As with Example 1, this inhibiting effect appears to be independent of the sunscreen effect as confirmed by the apparent lack of UV blocking even at a 100  $\mu$ mol genistein in water.

### Example 3

Ultraviolet B (UVB)-induced mRNA expression of protooncogenes *c-fos* and *c-jun* mRNA in the shaven skin of Sencar mice was characterized using the Northern hybridization. When mice were irradiated with the defined doses of UVB (5 and 15 kJ/m<sup>2</sup>), both *c-fos* and *c-jun* expression were induced in a time-dependent fashion. The level of *c-fos* and *c-jun* mRNA increased immediately and reached a maximum 1 h after UV irradiation. Expression of *c-fos* and *c-jun* appeared to be independent of UV dose.

Topical application of genistein (20  $\mu$ mol) 1 h prior to UV radiation substantially inhibited UVB-induced *c-fos* and *c-jun* expression induced by a low dose of UVB (5 kJ/m<sup>2</sup>). At a higher dose of UVB radiation (15 kJ/m<sup>2</sup>), genistein still substantially blocked UVB-induced *c-fos* expression, but had little effect on *c-jun* expression. The inhibition of UVB-induced protooncogene expression *in vivo* by genistein may be related to the signal transduction pathways because genistein was shown to downregulate UVB-induced tyrosine phosphorylation of epidermal growth factor receptor in cell culture, and mitogen protein kinases in mouse skin. The inhibitory effect of genistein on UV-induced protooncogene expression suggests its potential antipromotional role in photocarcinogenesis. The results of this experiment are shown in Figure 2. Lane 1 depicts no UV; Lane 2 depicts UV at a dosage level of 5 kJ/m<sup>2</sup> (no treatment); Lane 3 depicts 20  $\mu$ mol genistein applied one hour prior to 5 kJ/m<sup>2</sup> UV exposure; Lane 4 no UV; Lane 5 depicts 15 kJ/m<sup>2</sup> (no treatment); Lane 6 depicts 20  $\mu$ mol genistein applied one hour prior to exposure at a UV dosage 2 of 15 kJ/m<sup>2</sup>.

The results of this study were presented at the '96 Society of Investigative Dermatology in Washington, D.C., May 1-5, 1996. An abstract was published in Journal of Investigative Dermatology, 106(4): 856, 1996.

### Example 4

A procedure similar to that of Example 3 was followed except that the genistein (20  $\mu$ mol) was applied immediately after exposure to UVR (15 kJ/m<sup>2</sup>).

As shown in Figure 3, Lane 1: no UV + acetone; Lane 2: no UV + acetone; Lane 3: UV + acetone; Lane 4: UV + genistein. Thus even post-exposure treatment was shown to inhibit *c-fos* and *c-jun* expression.

#### Example 5

This experiment was reported in. Inhibitory effect of genistein on a tumor promotor-induced *c-fos* and *c-jun* expression in mouse skin, Wei et al., Oncology Reports 3:125-128, 1996.

Figure 4 shows that topical application of a promoting dose (8.5 nmol) of TPA significantly induces expression of *c-fos* and *c-jun* mRNA in mouse skin (lane 3) compared to the acetone-treated control (lane 1). As reported by Zwiller et al., Inhibition of PDGF-induced *c-jun* and *c-fos* expression by a tyrosine protein kinase inhibitor, Oncogene 6:219-221, 1991, there are two *c-jun* mRNA fragments (2.7 and 3.2 kb, respectively), which is due to the presence of two polyadenylation signals. Densitometric quantitation indicates that TPA significantly increases expression of these protooncogenes by 1.7-(*c-jun* 3.2 kb), 3.2-(2.7 kb *c-jun*), and 7.0-fold (*c-fos*), respectively, as compared to the acetone-treated control. Treatment of mouse with genistein alone slightly decreases the basal levels of *c-fos* and *c-jun* mRNA (lane 2; 10  $\mu$ mol genistein/acetone). However, pretreatment of mouse skin with genistein suppresses TPA-induced expression of both *c-fos* and *c-jun* (lane 4: 1  $\mu$ mol genistein/TPA; lane 5: 5  $\mu$ mol genistein/TPA; and lane 6: 10  $\mu$ mol genistein/TPA). Suppression of *c-fos* expression by genistein is more pronounced than that of *c-jun*, and at a dose of 10  $\mu$ mol genistein, TPA-induced *c-fos* expression is almost completely inhibited. Hybridization with a cyclophilin probe indicates that mRNA for the tested samples are equally loaded.

Figure 5 shows the quantitation of transcript levels of *c-jun* and *c-fos* from three independent experiments. All results were normalized by their corresponding cyclophilin intensity, and then versus the acetone treated control. The final results were expressed as the intensity ratio (treated groups vs. controls). The background intensity of acetone-treated control was  $3.2 \pm 4.9$  (*c-fos*),  $5.0 \pm 1.8$  (3.2 kb *c-jun*) and  $9.1 \pm 3.9$  (2.7 kb *c-jun*). Expression of both 3.2 kb and 2.7 *c-jun* mRNA

message is only weakly inhibited by about 20% at a high dose (10  $\mu$ mol) of genistein. In contrast, genistein strongly inhibits the TPA-induced expression of *c-fos* in a dose-dependent manner with an apparent  $IC_{50}$  of 6.5  $\mu$ mol genistein.

Figure 6 shows the effect of 10  $\mu$ mol genistein on TPA-induced *c-fos* expression at different dosing times. Genistein was topically applied to mouse skin 30 min. before, simultaneously or 30 min. after 5  $\mu$ g TPA treatment. Mice were sacrificed 2 h after TPA treatment and skin mRNA was purified. Protooncogene expression was analyzed by the Northern hybridization. A. *c-fos* and B, cyclophilin. Samples: Lane 1, acetone/acetone; lane 2, acetone/TPA; lane 3, 10  $\mu$ mol genistein applied 30 min before TPA; lane 4, 10  $\mu$ mol genistein applied simultaneously with TPA; and lane 5, 10  $\mu$ mol genistein applied 30 min after TPA. The results show that TPA significantly induces *c-fos* expression (lane 2) compared to acetone-treated control (lane 1). Genistein can significantly inhibit TPA-induced *c-fos* expression independent of the different dosing schedules (lanes 3-4).

#### Example 6

The various methods and compositions by which genistein may be topically applied are not limited by the present disclosure. The following is a representative list of suitable compositions:

- 1) 0.1-5  $\mu$ mol genistein/cm<sup>2</sup> in 5:95 DMSO:acetone.
- 2) 0.1-5  $\mu$ mol genistein/cm<sup>2</sup> in 30:70 propylene glycol:ethanol.
- 3) 0.1-5  $\mu$ mol genistein/cm<sup>2</sup> in 2:80 Tween 80:water.
- 4) 0.1-1  $\mu$ mol genistein/cm<sup>2</sup> in combination with para-aminobenzoic acid (to absorb UVB).
- 5) 0.1-1  $\mu$ mol genistein/cm<sup>2</sup> in combination with benzophenone derivatives (oxybenzone, dioxybenzone - to absorb UVB and UVA).
- 6) 0.1-1  $\mu$ mol genistein in combination with titanium dioxide and/or zinc oxide.

- 7) 0.1-1  $\mu\text{mol}$  genistein in combination with vitamins with antioxidant properties, such as vitamin A, vitamin C and vitamin E, including such vitamins in cosmetic moisturizing creams or skin care lotion, particularly for post-UV exposure.
- 8) 0.1-1  $\mu\text{mol}$  genistein in combination with other natural products, such as squalene from shark liver oil and aloe vera from liliaceae in cosmetic product.
- 9) 0.1-5  $\mu\text{mol}$  genistein added to low SPF sunblocker cream, now commercially available.
- 10) 0.1-5  $\mu\text{mol}$  genistein with alphahydroxy acids.
- 11) 0.1-5  $\mu\text{mol}$  genistein with Retin-A.
- 12) 0.1-5  $\mu\text{mol}$  genistein with betacarotene.

### CLAIMS

1. A method of inhibiting the harmful effect of UVR exposure to the human skin comprising topically applying a therapeutically effective amount of genistein to the skin at a time sufficiently close to the time of UVR exposure to inhibit UVR-induced damage to the skin.

2. A method according to claim 1, comprising applying genistein to the skin prior to exposure.

3. A method according to claim 2, comprising applying genistein to the skin within two hours prior to exposure.

4. A method according to claim 1, comprising applying genistein within two hours of exposure.

5. A method according to claim 1, comprising applying genistein in an amount of at least  $0.1 \mu\text{mol}/\text{cm}^2$  of skin.

6. A method according to claim 1, wherein the genistein is mixed with a carrier in a concentration of from  $0.1$  to  $1.0 \mu\text{mol}/\text{cm}^2$ .

7. A method according to claim 1, wherein the genistein is mixed with a composition having cosmetic or medicinal properties in a concentration of from  $0.1$  to  $1.0 \mu\text{mol}/\text{cm}^2$ .

8. A method according to claim 1, wherein the genistein is mixed with at least one of the following: dimethyl sulfoxide; dimethylsulfoxide:acetone; Tween 80; Tween 80:water; para-aminobenzoic acid; benzophenone derivatives; titanium dioxide, zinc oxide; antioxidant vitamins including vitamins A, C and E; cosmetic moisturizing cream, skin care lotions.

squalene; aloe vera; sunblock cream; lipid; alphahydroxy acids; Retin-A; betacarotene.

9. A method according to claim 1 of mitigating the cancer-inducing effect of UVR, comprising topically applying an amount of genistein sufficient to inhibit UVR-induced skin photocarcinogenesis.

10. A method according to claim 1 of inhibiting the skin photoaging effect of UVR, comprising topically applying an amount of genistein sufficient to inhibit UVR-induced aging.

1/4

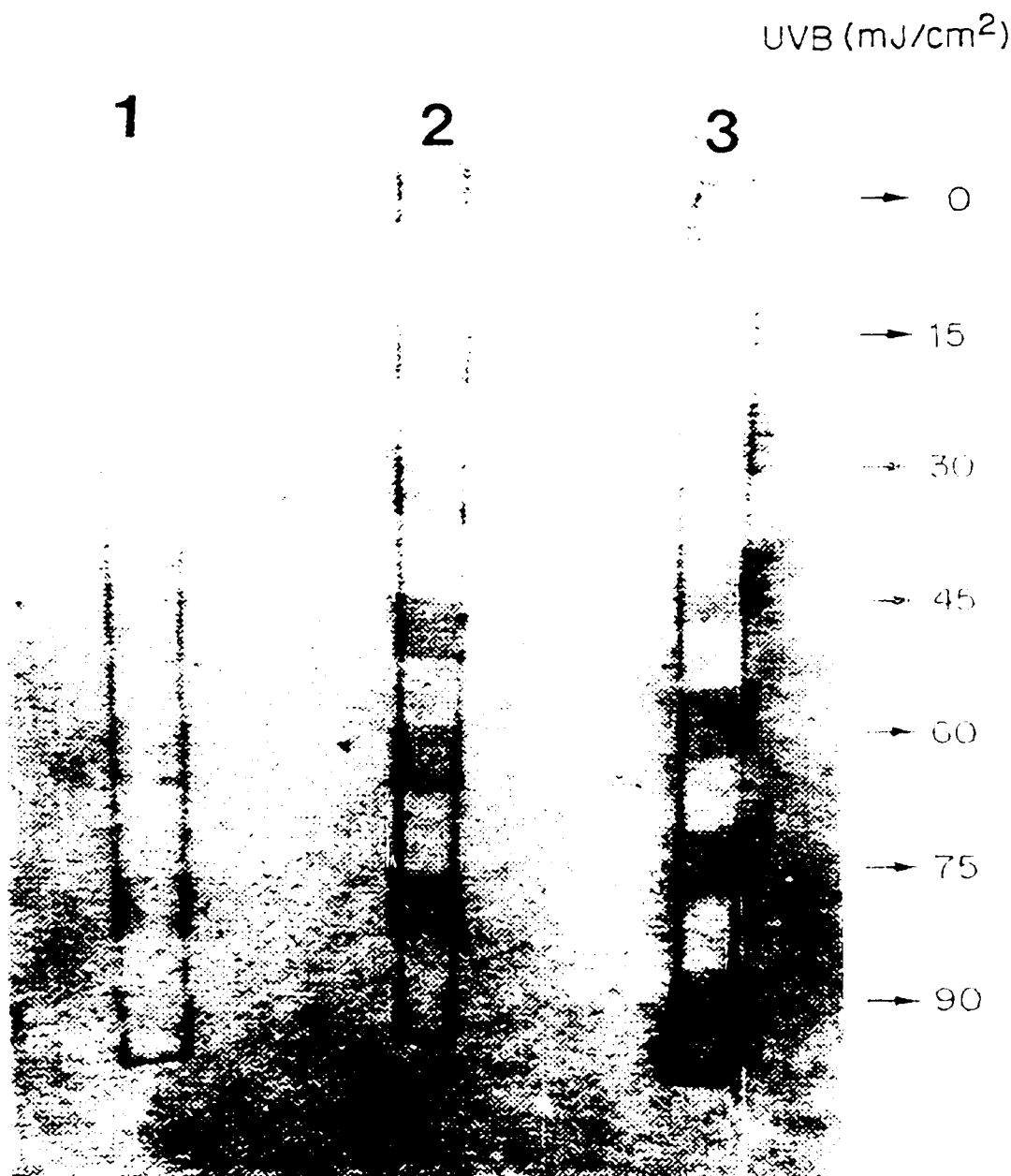


FIG. 1

2/4



FIG. 2

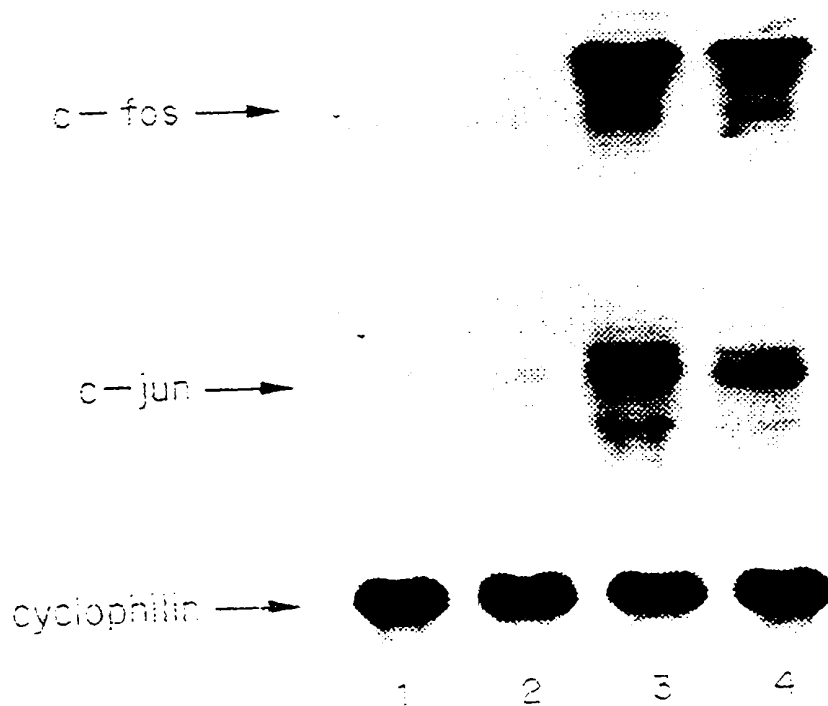
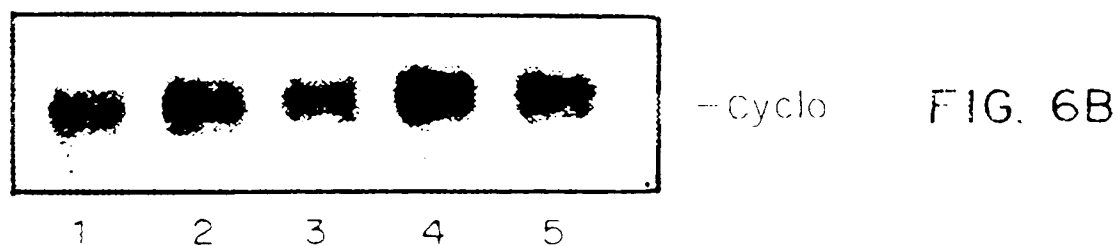
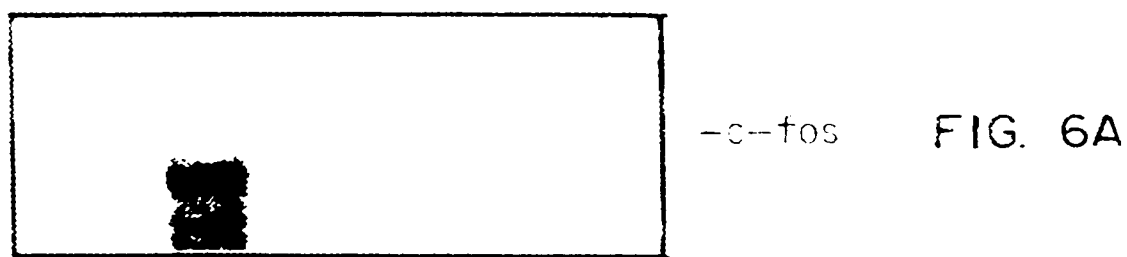
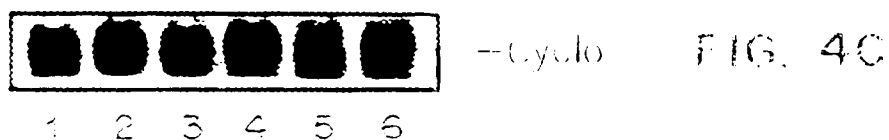
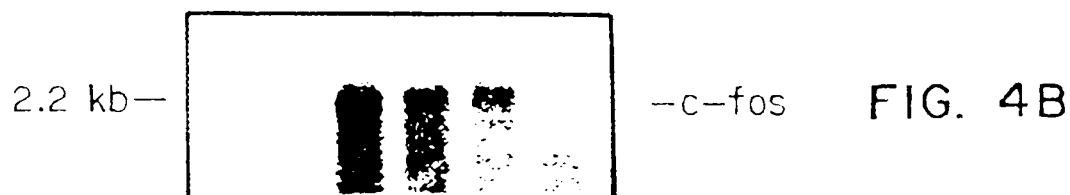
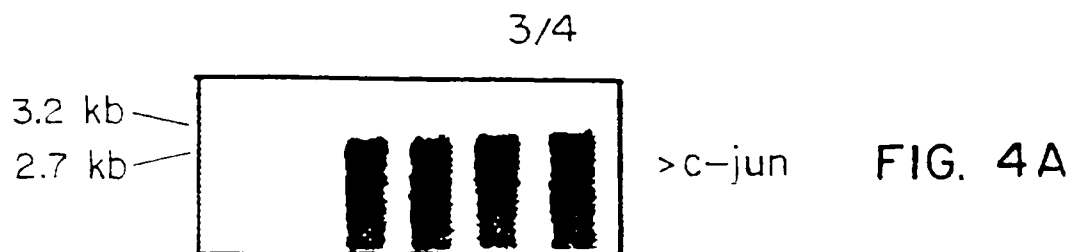


FIG. 3

SUBSTITUTE SHEET (RULE 26)



SUBSTITUTE SHEET (RULE 26)

4/4

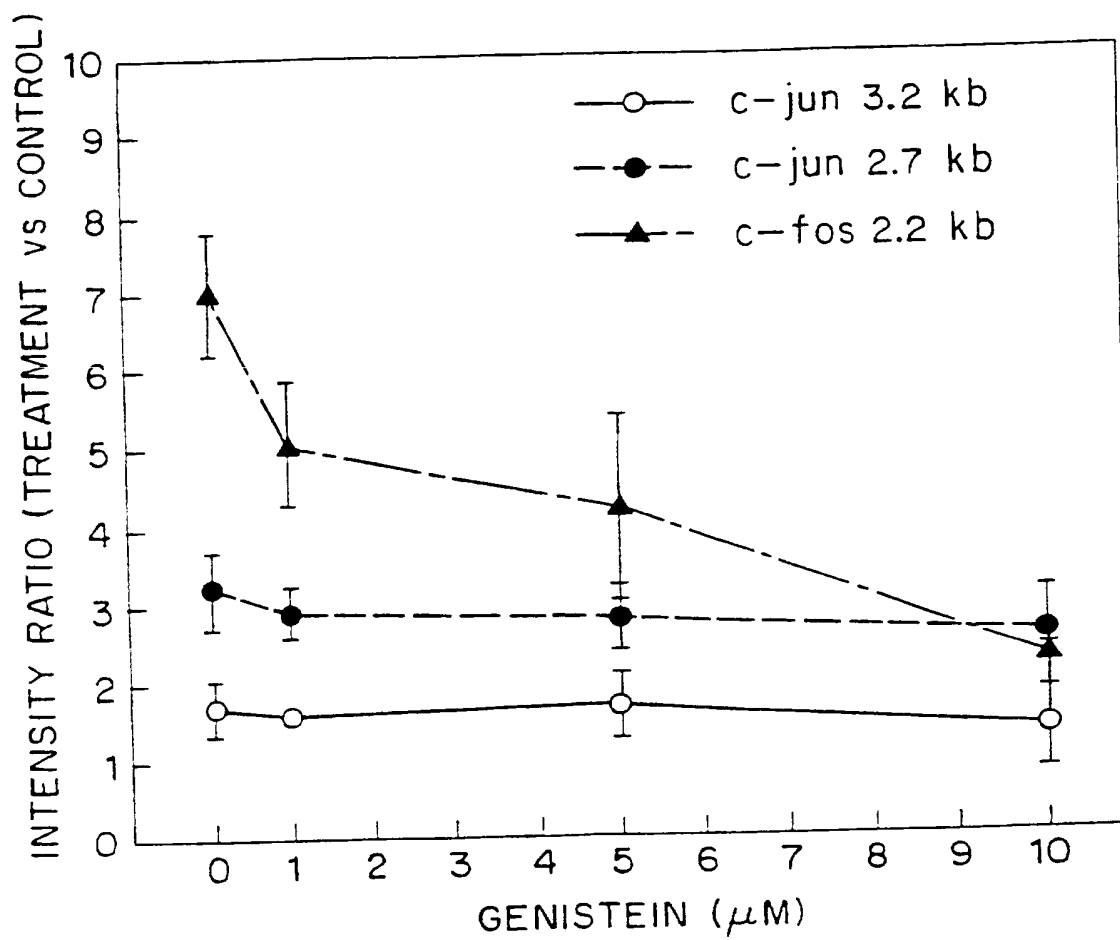


FIG. 5

SUBSTITUTE SHEET (RULE 26)

